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Claims

1. A method for establishing whether at least one target polynucleotide is present in a sample, comprising the steps of

i) providing a sample to be analysed for the presence of the at least one target polynucleotide,

ii) adding at least one polynucleotide probe at least partly complementary to a sub-sequence of the at least one target polynucleotide, wherein the at least one probe comprises at least one detectable label,

iii) incubating the sample under conditions suitable for the formation of at least one hybrid polynucleotide comprising the at least one probe and the at least one target polynucleotide, when present,

iv) recording spectral data from an environment comprising at least part of the sample,

v) analysing the spectral data, and

vi) establishing whether the target polynucleotide is present.

2. The method according to claim 1, wherein the analysis of the spectral data can distinguish for each of the at least one probe whether the probe is part of the at least one hybrid polynucleotide or not part of the at least one hybrid polynucleotide.

3. The method according to claim 1 or 2, wherein the analysis of the spectral data can distinguish for each of the at least one probe, when the probe is part of the at least one hybrid polynucleotide, whether or not there is a mismatch between the probe and the sub-sequence of the at least one target polynucleotide.

4. The method according to any of claims 1 to 3, wherein the spectral data are analysed using multivariate analysis.

5. The method according to any of the preceding claims 1 to 4, wherein the at least one probe has a length of 6 to 50 nucleotides, preferably 6 to 25 nucleotides, such as 6 to 8 nucleotides, 8-10 nucleotides, 10-12 nucleotides, 12-14 nucleotides, 14-16 nucleotides, 16-18 nucleotides, 18-20 nucleotides, 20-22 nucleotides, or 22-25 nucleotides.

6. The method according to claim 5, wherein the sequence complementarity between target and probe in a range of overlap is at least 50%, more preferably at least 60 %, more preferably at least 70 %, more preferably at least 80 %, more preferably at least 85 %, more preferably at least 90 %, more preferably at least 95 %, more preferably at least 96 %, more preferably at least 98 %, more preferably 100%.

7. The method according to any of the preceding claims, wherein at least one probe comprises at least one RNA monomer.

8. The method according to any of the preceding claims, wherein at least one probe comprises at least one DNA monomer.

9. The method according to any of the preceding claims, wherein at least one probe comprises at least one PNA monomer.

10. The method according to any of the preceding claims, wherein at least one probe comprises at least one methylated monomer.

11. The method according to any of the preceding claims, wherein at least one probe comprises at least one LNA monomer.

12. The method according to any of the preceding claims, wherein at least one probe comprises a mixture of monomers in claims 7-11.

13. The method according to any of the preceding claims, wherein one probe is capable of hybridising to two or more target polynucleotides.

14. The method according to any of the preceding claims, comprising using at least two polynucleotide probes capable of hybridising to two different target polynucleotides.

15. The method according to any of claims 1 to 12, comprising using at least two polynucleotide probes capable of hybridising to the same target polynucleotide.

16. The method according to claim 14 or 15, wherein the two probes are linked to identical or different detectable labels.

17. The method according to any of the claims 1 to 12, comprising using at least three probes capable of hybridising to three different target polynucleotides.

18. The method according to any of claims 1 to 12, comprising using at least three probes capable of hybridising to one or two different target polynucleotides

19. The method according to claim 17 or 18, wherein the three probes are linked to identical or different detectable labels.

20. The method according to any of claims 1 to 12, comprising using at least four oligonucleotide probes capable of hybridising to four different target polynucleotides.

21. The method according to any of claims 1 to 12, comprising using at least four oligonucleotide probes capable of hybridising to one, two or three different target polynucleotides.

22. The method according to claim 20 or 21, wherein the four labels are linked to identical or different detectable labels.

23. The method according to any of claims 1 to 12, comprising using five or more probes capable of hybridising to different target polynucleotides.

24. The method according to any of the preceding claims, wherein at least one probe comprises a probe selective for apolipoprotein B mutations related to atherosclerosis.

25. The method according to claim 24, wherein the probe comprises a sequence from any of SEQ ID NO 1 to 4 or similar probes, where the mutation/polymorphism is placed in the 5' or 3' end.

26. The method according to any of the preceding claims, wherein at least one probe is selective for apolipoprotein E polymorphism (apoE2, E3 and E4) related to neurological diseases.

27. The method according to claim 26, wherein the probe comprises a sequence from any of SEQ ID NO 5 to 8 or similar probes, where the mutation/polymorphism is placed in the 5' or 3' end.

28. The method according to any of the preceding claims, wherein at least one probe is selective for human muscle glycogen synthase polymorphism related to diabetes mellitus.

29. The method according to claim 28, wherein the probe comprises a sequence from any of SEQ ID NO 9 to 10 or similar probes, where the mutation/polymorphism is placed in the 5' or 3' end.

30. The method according to any of the preceding claims, wherein at least one probe is selective for methylene tetrahydrofolate reductase polymorphism related to osteoporose.

31. The method according to claim 30, wherein the probe comprises a sequence from any of SEQ ID NO 13 to 14 or similar probes, where the mutation/polymorphism is placed in the 5' or 3' end.

32. The method according to any of the preceding claims, wherein at least one probe is selective for Dnase1 mutations related to rheumatological diseases.

33. The method according to claim 32, wherein the probe comprises a sequence from any of SEQ ID NO 11 to 12 or similar probes, where the mutation/polymorphism is placed in the 5' or 3' end.

34. The method according to any of the preceding claims, wherein at least one probe is selective for a mutation in the BRCA1 gene or in the BRCA2 gene.

35. The method according to claim 34, where the probe comprises a nucleotide sequence selected from any of SEQ ID No 27-30 or similar probes, where the mutation/polymorphism is placed in the 5' or 3' end.

36. The method according to any of the preceding claims, wherein at least one probe is selective for mismatch repair gene mutations related to cancer.

37. The method according to claim 36, wherein the probe comprises a sequence from any of SEQ ID NO 15 to 16.

38. The method according to any of the preceding claims, wherein at least one probe is selective for a mutation in a promoter sequence or other expression signal.

39. The method according to any of the preceding claims, wherein at least one probe is selective for a mutation in a coding sequence (exons) and the intervening introns.

40. The method according to any of the preceding claims, wherein at least one probe is selective for a microbial target nucleic acid sequence.

41. The method according to claim 40, wherein the probe is selective for a microbial 16S, 18S, or 23S rRNA sequence.

42. The method according to claim 41, wherein the probe has a nucleotide sequence selected from SEQ ID NO 17 to 19.

43. The method according to any of the preceding claims, wherein at least one target polynucleotide comprises RNA, such as mRNA and/or rRNA and or tRNA

44. The method according to claim 43, wherein the rRNA comprises 5S, 5.5-5.8S, 16S, 18S, 23S, 25-28S rRNA.

45. The method according to any of the preceding claims, wherein at least one target polynucleotide comprises DNA.

46. The method according to claim 45, wherein the DNA is selected from the group comprising genomic DNA, organelle DNA, mitochondrial DNA, chloroplast DNA, cDNA, environmental DNA, virus DNA.

47. The method according to any of the preceding claims, wherein at least one target polynucleotide comprises a synthetic polynucleotide sequence.

48. The method according to any of the preceding claims, further comprising inclusion of various control polynucleotides in the hybridisation mixture, such as positive controls (wild-type, mutation, heterozygote), negative control (dummy DNA sequence).

49. The method according to any of the preceding claims, wherein the target polynucleotide comprises chemically or biologically modified nucleic acids.

50. The method according to claim 49, wherein the modification comprises modification of cytosine by bisulphite.

51. The method according to any of claims 43 to 50, wherein the target polynucleotide comprises a mixed polymer of any of the polymers of claims 43 to 50.

52. The method according to any of the preceding claims, wherein at least one target polynucleotide has a length of 8 bases to 1000 kb.

53. The method according to claim 52, wherein the length of at least one target polynucleotide is from 8-15 bases, from 15-30 bases, from 30 to 50 bases, from 50 to 100 bases, from 100 to 200 bases, from 200 to 300 bases, from 300 to 500 bases, from 500 to 750 bases, from 750 to 1000 bases, from 1000 to 1500 bases, from 1500 to 3000 bases, from 3000 to 5000 bases, from 5000 to 10000 bases, from 10000 to 15000 bases, from 15000 to 20000 bases, from 20000 to 25000, from 25000 to 30000 bases, from 30000 to 35000 bases, from 35000 to 40000 bases, from 40000 to 45000 bases, from 45000 to 50000 bases, from 50000 to 75000 bases, from 75000 to 100000 bases, from 100 kb to 250 kb, from 250 to 500 kb, from 500 kb to 750 kb, from 750 kb to 1000 kb, or more than 1000 kb.

54. The method according to any of the preceding claims, wherein the length of the overlap between the probe and target polynucleotide is at least 5 nucleotides, more preferably at least 6 nucleotides, such as at least 7 nucleotides, for example 8 nucleotides, such as at least 9 nucleotides, for example at least 10 nucleotides, such as at least 15 nucleotides, for example at least 20 nucleotides, for example at least 25 nucleotides, such as at least 50 nucleotides, for example at least 100 nucleotides.

55. The method according to any of the preceding claims, wherein the length of at least one probe is 7 to 1000 nucleotides, such as from 7 to 10 nucleotides, 10 to 15 nucleotides, 15 to 20 nucleotides, 20 to 25 nucleotides, 25 to 35 nucleotides, 35 to 50 nucleotides, 50 to 75 nucleotides, 75 to 100 nucleotides, 100 to 150 nucleotides, 150 to 200 nucleotides, 200 to 250 nucleotides, 250 to 350 nucleotides, 350 to 500 nucleotides, 500 to 750 nucleotides, 750 to 1000 nucleotides, or above 1000 nucleotides.

56. The method according to any of the preceding claims, wherein the nucleotide being complementary to a polymorphism/mutation in a target polynucleotide is positioned in the 3' or 5' terminal of the probe.

57. The method according to any of the preceding claims 1 to 55, wherein the nucleotide being complementary to a polymorphism/mutation in a target polynucleotide is positioned in the centre of the probe.

58. The method according to any of the preceding claims 1 to 55, wherein the nucleotide being complementary to a polymorphism/mutation in the target polynucleotide is positioned at least 1 nucleotide from the 3' or 5' terminal, such as at least 2 nucleotides from the 3' or 5' terminal, for example at lest 3 nucleotides from any of said terminals, such as at least 5 nucleotides from any of said terminals, for example at least 10 nucleotides from any of said terminals.

59. The method according to any of the preceding claims 1 to 55, wherein the probe comprises a sequence which is complementary to the sequence lying immediately upstream or immediately downstream to a polymorphic site in the target polynucleotide and the probe does not contain a nucleotide being complementary to the polymorphic site.

60. The method according to any of the preceding claims, wherein at least one label is bound to the 3' or 5' terminal nucleotide of the probe.

61. The method according to any of the preceding claims, wherein at least one label is bound to a non-terminal nucleotide of the probe.

62. The method according to any of the preceding claims, wherein at least one label is bound to the nucleotide being complementary to the polymorphic site.

63. The method according to any of the preceding claims, wherein at least one label is bound to a nucleotide at least 1 nucleotide upstream or downstream to the nucleotide complementary to the polymorphic site, such as at least 2 nucleotides upstream or downstream, for example at least 3 nucleotides, such as at least 5 nucleotides, for example at least 10 nucleotides.

64. The method according to any of the preceding claims, wherein at least one probe has at least two stretches of complementarity to at least one target polynucleotide, such as at least 3 stretches, for example at least 4 stretches, such as at least 5 stretches.

65. The method according to claim 64, wherein two stretches are separated by a nucleotide sequences, which does not hybridise to the target polynucleotide.

66. The method according to any of the preceding claims, further comprising amplification of a polynucleotide prior to hybridisation.

67. The method according to claim 66, wherein the amplification comprises PCR, long range PCR, and any variant of PCR amplification.

68. The method according to claim 66, wherein the amplification comprises ligase chain reaction, asymmetric amplification, single-strand amplification, T7 amplification, NASBA (Nucleic Acid Sequence-Based Amplification), strand displacement amplification, or rolling circle amplification, or T7 polymerase amplification.

69. The method according to claim 66, wherein the amplification comprises amplification in bacteria, yeast, other cells, YAC amplification, BAC amplification or other artificial chromosome based amplifications.

70. The method according to claim 66, wherein the amplification comprises allele specific amplification.

71. The method according to any of the preceding claims, wherein undesired hybridisation reactions are prevented by the addition of one or more helper polynucleotides capable of hybridising to the target polynucleotide at a sub-sequence which does not overlap with the sub-sequence to which the probe hybridises.

72. The method according to any of the preceding claims, wherein prior to the hybridisation, a step aimed at generating single stranded polynucleotides is performed.

73. The method according to any of the preceding claims, wherein the formation of a hybrid polynucleotide takes place under conditions of

a) optimal or suboptimal stringency providing sufficient stable complexes for discriminatory signal detection,

b) any composition of buffers optimising discriminatory signal detection,

c) any form and concentrations of one or more salts optimising discriminatory signal detection,

d) any additives including but not limited to stabilisers and/or quenchers optimising discriminatory signal detection,

e) temperature range for hybridisation specific for any specific combination of analyte and probe optimising discriminatory signal detection, and/or

f) any range of time of hybridisation necessary to optimise discriminatory signal detection.

74. The method according to any of the preceding claims, wherein the formation of a hybrid is performed at a temperature between 10 and 90°C such as 10 to 20 °C, 20-30 °C, 30 to 40 °C, 40 to 50 °C, 50 to 60 °C, 60 to 70 °C, 70 to 80°C, or 80 to 90°C.

75. The method according to any of the preceding claims, wherein the formation of a hybrid is performed in a buffer, which is a PCR buffer, and/or which is non-fluorescent, and/or which stabilises the spectrum of electromagnetic radiation, and/or which allows hybridisation.

76. The method according to any of the preceding claims, wherein hybridisation is carried out under conditions of high stringency allowing hybridisation only between perfect matches.

77. The method according to any of the preceding claims, wherein hybridisation is carried out under conditions of medium to high stringency allowing hybridisation between probe and target in the presence of one or more mismatches.

78. The method according to any of the preceding claims, wherein hybridisation is carried out in solution.

79. The method according to any of the preceding claims, wherein the target or the probe is linked to a solid support prior to hybridisation.

80. The method according to claim 79, wherein said solid support comprises beads such as magnetic beads and/or the surface of a well.

81. The method according to any of the preceding claims, wherein at least one probe hybridises only to one target polynucleotide.

82. The method according to any of the preceding claims, wherein at least one probe hybridises to both a wild-type target polynucleotide and to a target polynucleotide carrying a mutation or polymorphism.

83. The method according to any of the preceding claims, wherein the at least one detectable label comprises a fluorescent label.

84. The method according to claim 83, wherein the label is selected from the list in table 2 and 3.

85. The method according to any of the preceding claims, wherein the at least one label comprises a phosphorescent label.

86. The method according to any of the preceding claims, wherein the at least one label comprises a chromogenic label such as TMB (3,3',5,5-tetramethylbenzidine).

87. The method according to any of the preceding claims, wherein recording spectral data comprises detection of signal for at least 10 discrete wavelengths, more preferably at least 20 discrete wavelengths, more preferably at least 50 discrete wavelengths, more preferably at least 100 discrete wavelengths, such as at least 200 discrete wavelengths, for example at least 250 discrete wavelengths, such as at least 300 discrete wavelengths, for example at least 400 discrete wavelengths, such as at least 500 discrete wavelengths, for example at least 600 discrete wavelengths, such as at least 750 discrete wavelengths, for example at least 1000 discrete wavelengths, such as at least 1250 discrete wavelengths, for example at least 1500 discrete wavelengths, such as at least 2000 discrete wavelengths.

88. The method according to claim 87, wherein the distance between the discrete wavelengths is 10 nm or less, more preferably 5 nm or less, more preferably 3 nm or less, more preferably 2 nm or less, more preferably 1 nm or less, such as 0.8 nm, for example 0.75 nm, such as 0.7 m, for example 0.6 nm, such as 0.5 nm, for example 0.25 nm, such as 0.1 nm, for example 0.05 nm or less, such as 0.01 nm or less.

89. The method according to any of the preceding claims, wherein the spectral data recorded comprises a fluorescence spectrum between 180 and 950 nm.

90. The method according to claim 89, wherein the fluorescence spectrum is an excitation spectrum.

91. The method according to claim 89, wherein the fluorescence spectrum is an emission spectrum.

92. The method according to any of the preceding claims, further comprising recording of spectral data from the polynucleotide probe alone.

93. The method according to any of the preceding claims, further comprising recording spectral data from the hybrid polynucleotide and from a polynucleotide probe alone and/or, from a non-hybridising polynucleotide probe contacted by the target polynucleotide, and/or from a polynucleotide probe contacted with a non-hybridising polynucleotide sequence.

94. The method according to any of the preceding claims, wherein the correlation to presence or absence of the hybrid comprises multivariate analysis.

95. The method according to claim 94, wherein multivariate analysis comprises general multivariate analysis, principal component analysis and extensions of this, exploratory and confirmatory factor analysis in its various forms, Cluster and latent class analysis including scaled latent class analysis, structural equation analysis, Fixed mixture analysis and combinations hereof.

96. The method according to any of the preceding claims, wherein data are treated using a neural network.

97. The method according to any of the preceding claims, wherein spectral data are recorded from hybrid polynucleotides in solution.

98. The method according to claim 97, wherein the spectral data are recorded from a solution comprising both the hybrid polynucleotide and unhybridised probe.

99. The method according to any of the preceding claims 1 to 96, wherein spectral data are recorded from hybrid polynucleotides bound to a solid support.

100. The method according to claim 99, wherein the solid support comprises a solid surface capable of immobilising a capture probe, a capture probe capable of immobilising the target polynucleotide, and a labelled detection probe capable of hybridising to the immobilised target polynucleotide.

101. The method according to claim 99, wherein the solid support is a disposable or reusable device such as but not exclusively a flow-through system.

102. The method according to claim 99, wherein the capture probe is immobilised a priori to the solid surface.

103. The method according to claim 99, wherein the capture probe is hybridised to the target before immobilisation on a solid support.

104. The method according to claim 99, wherein the capture probe(s) is/are (an) allele specific probe(s).

105. The method according to any of the preceding claims 1 to 96, wherein spectral data are recorded from hybrid polynucleotides in a gas phase.

106. The method according to any of the preceding claims 1 to 96, wherein spectral data are recorded from hybrid polynucleotides in vacuum.

107. The method according to any of the preceding claims, wherein the spectral data are recorded via mass spectroscopy.

108. The method according to any of the preceding claims, further comprising the step of determining the presence or absence of a mutation or polymorphism in the genome of an individual on the basis of the information obtained concerning the presence or absence of the at least one target polynucleotide.

109. The method according to any of the preceding claims, further comprising the step of diagnosing a disease or health related state or determining a genetic predisposition of an individual on the basis of the information obtained concerning the presence or absence of the at least one target polynucleotide.

110. A kit for detection of a mutation or a polymorphism comprising

at least one oligonucleotide probe capable of hybridising to a preselected region of a target polynucleotide, the polynucleotide probe further comprising at least one detectable label,

instructions enabling correlation of spectral data recorded from a hybrid polynucleotide between said at least one oligonucleotide probe and said target polynucleotide to the presence or absence of said mutation or polymorphism using multivariate analysis.

111. The kit according to claim 110, wherein the instructions are in the form of calibration data on a data carrier, such as floppy disc, a CD-ROM, a DVD, ROM, chips, memory-cards, bar-codes.

112. The kit according to claim 110, wherein the instructions are in the form of the address of a propagated signal comprising calibration data, which can be transferred over a network, such as e-mail, internet, on-line nets, fibre-optics, power-cables, satellite-dishes.

113. The kit according to claim 110, wherein the instructions are in the form of calibration data which can be entered into a computer unit.

114. The kit according to claim 110, further comprising at least one control polynucleotide capable of hybridising to the oligonucleotide probe and non-hybridising polynucleotide(s)

115. The kit according to claim 110, being in the form of a tube container with at least one probe linked to the inner surface, being a solid surface, the tube wall allowing electromagnetic radiation to pass the walls.

116. The kit according to claim 115, wherein the tube comprises more than one probe linked to more than one location, the locations being spatially separate.

117. The kit according to claim 115, wherein the tube comprises more than one probe the probes having detectably different labels.

118. A system for establishing whether at least one target polynucleotide is present in a sample, comprising

vii) at least one polynucleotide probe being at least complementary to a target polynucleotide, the probe comprising a detectable label,

viii) a sample chamber from which electromagnetic radiation can be recorded,

ix) a source of spectrally resolved electromagnetic radiation,

x) means for sensing and recording a spectrum of electromagnetic radiation from the sample chamber, and

xi) a computer unit for storing spectral data of electromagnetic radiation and having instructions to treat the recorded spectral data using multivariate analysis.

119. A system for detection of a hybrid polynucleotide comprising

i) at least one oligonucleotide probe being at least partly complementary to a target polynucleotide, the probe comprising a detectable label,

ii) a sample chamber from which electromagnetic radiation can be recorded,

iii) a source of spectrally resolved electromagnetic radiation,

iv) means for sensing and recording a spectrum of electromagnetic radiation from the sample chamber, and

v) a computer unit for storing spectral data of electromagnetic radiation and having instructions to treat the recorded spectral data using multivariate analysis.

120. The system according to claim 118 or 119, further comprising a computer controlled robot to transfer solutions to the sample chamber.

121. The system according to claim 118 or 119, further comprising means to control the temperature of the sample chamber.

122. The system according to claim 118 or 119, wherein the sample chamber is in the form of a tube with at least one probe linked to the inner surface.

123. The system according to claim 122, wherein the tube comprises more than one probe linked to more than one spatially separate location and the system comprises means to record a spectrum from each of the spatially separate locations.

124. The system according to claim 122, wherein the tube comprises more than one probe, the more than one probe having detectably different labels.

125. The system according to claim 118 or 119, being adapted to accommodate a multi-well dish and record a spectrum for each well.

126. The system according to claim 125, being adapted to accommodate a 96 well dish.

127. The system according to claim 125, being adapted to accommodate a 384 well or more dish.

128. The system according to claim 125, being adapted to accommodate a solid support such as but not exclusively a dish or a rod.

129. The system according to claim 125, being adapted to accommodate spinning dishes or rotating and displaceable rods.

130. The system according to any of claims 118 to 129, wherein the sample chamber further comprises means for immobilisation of one or more target polynucleotides.